SUPPLEMENTAL DIGITAL CONTENT

Immunohistochemical Techniques for FSH and LH Analysis of Pituitary Tumors

Four-micron thick sections of formalin fixed tissue were used for immunoperoxidase analysis after baking at 60º C for 1 hour, deparaffinization, and rehydration (100% xylene X4 for 3 minutes each, 100% ethanol X4 for 3 minutes each, and running water for 5 minutes). The sections were blocked for peroxidase activity with 3% hydrogen peroxide in methanol for 10 minutes and washed under running water for 5 minutes. Then sections were digested with Protease type XXIV, bacterial (Sigma Chemical Co., St. Louis, MO at 0.01% in pre-warmed 0.005M Tris Buffer Saline, pH 7.6) at 37° C for 10 min. The sections were incubated with primary antibody either for FSH (DAKO, cat# M3504, 1:30) or LH (DAKO cat# M3502, 1:100), 40 min in room temperature, followed by incubation with secondary antibody (DAKO Envision Mouse, K4007) for 30 min. All the incubations were carried out in a humid chamber at room temperature. The slides were rinsed with PBS in between incubations. The sections were developed using 3,3'-diaminobenzidine (DAB) (Sigma Chemical Company) as substrate and counter-stained with Mayer's Hematoxylin.